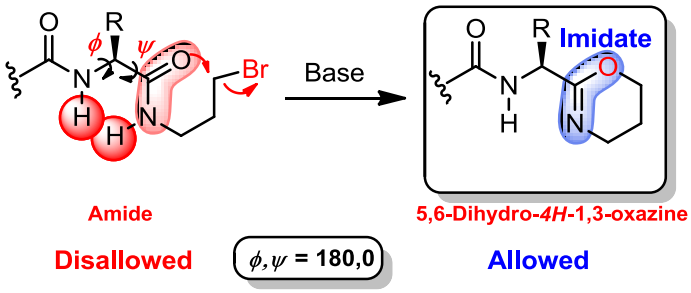
This thesis entitled **"Design and Access to Disallowed and Unusual Conformers of Peptides in Crystals and in Solution: Structural Consequences of the Imidate and Thioimidate Isosteres for the Peptide bond"** is divided into eight chapters.

Chapter 1: Section A: Design of Disallowed Folds by Employing an Amide to Imidate Modification

The range of disallowed dihedral angles (ϕ, ψ) for residues in peptides is governed by their local steric and electrostatic clashes. Rare tolerances of violations in these angles are attributed to distortions in both local and global bond characteristics of the peptides. Discerning the origins of such disallowed angles and the consequent distortions in body of the peptides is essential, for a complete understanding of the protein fold, to improve the crystal database for validation of rare but acceptable residue conformations and for validation and improvement of theoretical models that evaluate the interactions that define the Ramachandran space. Unlike for the ordered secondary structures such as β-sheets α-helices and β-turns currently there are no models for residues constrained in disallowed folds. We reasoned that **residues may be stabilized in disallowed folds in peptides if a neighbouring group and hence its local unfavorable clashes can be selectively modified to a motif that favors such space**.



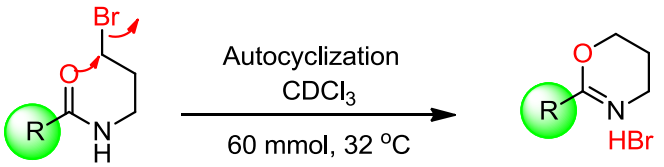
Steric clashes of the type H•••$X_{i\pm n}$ involving the backbone amide hydrogen (H) contribute to ~60% of disallowed ϕ,ψ space. Conversion of an amide to an imidate (A→I) will remove the corresponding H and hence the steric clashes related to it in peptides. Importantly, this will introduce an H-bond acceptor N (of imidate) in place of an H-bond donor NH (of amide), which will allow formation of unusual H-bonding interactions between the imidate N and the neighbouring Hs and hence constrain residues in otherwise inaccessible dihedral angles. The conversion of A→I is challenging owing

to difficulties in selective synthesis, stability and purification of the imidate motif. We address all these concerns by the selective conversion of a backbone amide in peptides to the relatively stable cyclic 5,6-dihydro-*4H*-1,3-oxazine imidate isostere, by an intra-molecular nucleophilic cyclo-*O*-alkylation reaction.

Chapter 1: Section B: Autocyclo-*O*-Alkylation of *N*-(3-Bromopropyl)amides into 2-Alkyl-5,6-Dihydro-4*H*-1,3-Oxazinehydrobromides

We are describing the reactivity of *N*-(3-bromopropyl)amides that are precursors for 2-peptide-5,6-dihydro-4*H*-1,3-oxazine. The starting materials, 3-bromopropylamides, were synthesized in good yields by coupling the corresponding carboxylic acids and anhydrides with 3-bromopropylaminehydrobromide using standard mixed anhydride peptide coupling protocol. *N*-(3-bromopropyl)-acylamides are unstable during the isolation.

Time-dependent ^{1}H NMR of all the acetamides revealed that they underwent clean auto-cyclization to form the corresponding 2-alkyl-5,6-dihydro-4*H*-1,3-oxazine hydrobromides following first order rate. The salts were easily isolated in high purity by trituration of the mixtures with ether.



S.No.	R	$t_{1/2}$ (h)	Reaction rate (10^{-7} sec^{-1})	Reaction Monitor time (h)	% of conversion
1	H	-	-	100	0
2	Me	36[a]	52.4	103	86[b]
3	Et	25[a]	75.5	88	91[b]
4	^{i}Pr	23[a]	84.4	55	81[b]
5	^{t}Bu	19[a]	100.8	34	74[b]

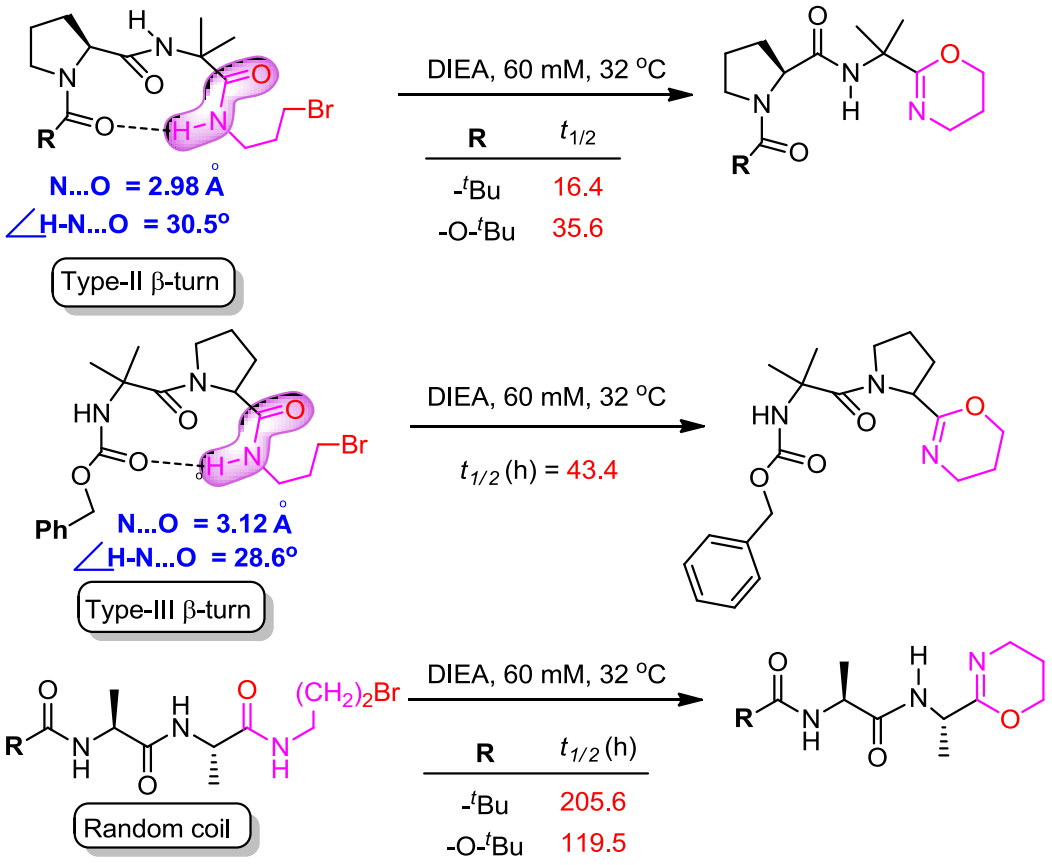
[a] $t_{½}$ were calculated from time dependent ^{1}H NMR studies;
[b] isolated yields after ether wash;

The $t_{1/2}$ of autocycliation of decreased upon increase in electron density on the R-carbon. Notably, the *tert*-butyl substituent cyclized significantly faster than acetamide which have enolizable hydrogens at the R-carbon. Thus, the cyclization rate is

affected predominantly by the inductive effect of the R-carbon substituents. The formamide remained stable and unchanged due to the poor electron-donating ability of hydrogen.

Chapter 1: Section C: Intramolecular Hydrogen Bond Assistance Improves Autocyclization in *N*-(3-Bromopropyl)amides

The autocyclisation do not go to 100% completion. This is because the released hydrobromic acid quenches the nucleophilicity of amide carbonyl oxygen. In order to scavenge hydro bromic acid, we used 1 equivalent of DIEA base is acting only acid scavenger which conformed by unaffecting the reaction rate upon increasing equivalents of DIEA.

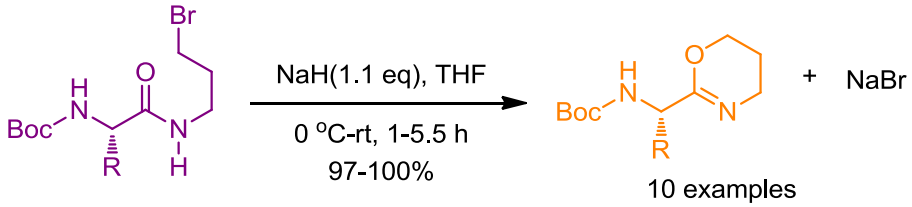


We found that autocyclisation of *N*-(3-bromopropyl)amides rates in peptides involved in intramolecular backbone H-bonding interactions improve the autocyclization rates significantly than unstructured (random coil) peptides. Even with in the ordered structures the rate depends on the proximity of H-bonding distances as well as the H-bond acceptor strength. Half-life of autocyclisation in various peptide secondary

structures are determined from time variant ^{1}H NMR studies performed at 60 mM peptide concentration in $CDCl_3$ at 32 ^oC.

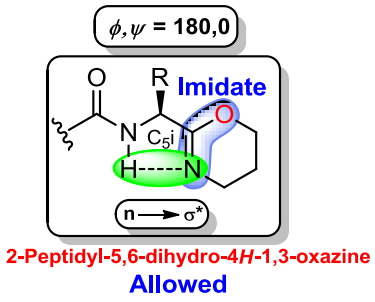
Chapter 2: Section A: Synthesis and Isolation of 5,6-Dihydro-4*H*-1,3-Oxazine Containing Peptidomimetics

We have introduced 5,6-Dihydro-4*H*-1,3-oxazine as the imidate isostere at C-terminus of a number of peptides through NaH (base) mediated intramolecular cyclo-*O*-alkylation of *N*-(3-bromopropyl)amides. The amide to imidate (A→I) modification reaction is faster (1-5.5 h), Exhibiting no electronic and structural effects under these conditions. The side product NaBr can be easily separated by filtration through celite. No side products were observed and there is no need of further purification to get pure 1,3-oxazines in quantitative yields in all the peptidomimetics. Using this synthetic protocol we have synthesised a variety of 1,3-oxazine containing peptide analogues including aliphatic, branched aliphatic, polar side chains and larger peptides. We show that the retention of configuration at C^{α} of peptides during the base mediated cyclo-*O*-alkylation reaction.



Chapter 2: Section B: 1,3-Oxazine forms Strong *n* → *σ Interactions in Peptides**

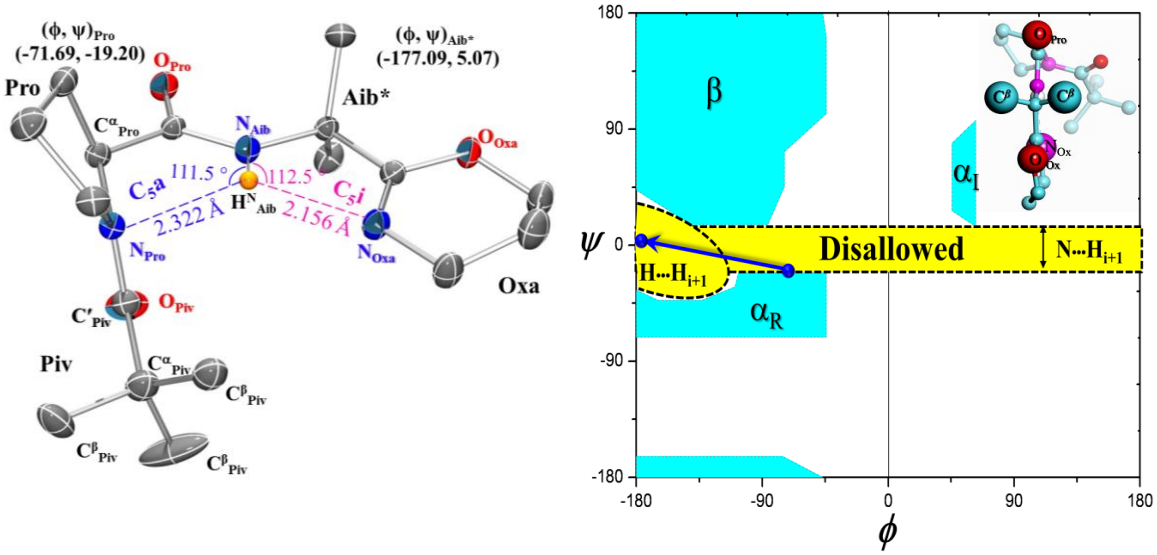
We presented spectroscopic evidence for the formation of strong 5-membered intramolecur hydrogen bonding interactions (C_5i structure) between imidate nitrogen and preceding amide H in 5,6-dihydro-4*H*-1,3-oxazine analogues peptides. Based on ^{1}H NMR chemical shift data of the oxazine containing peptidomimetics. We show that the C_5i structures are more populated at Aib due to operation of the Thorpe-Ingold effect.



The strength of hydrogen bonding interaction in C_5i structure is similar to those of the highly buried backbone hydrogen bonding interaction found in the middle of a model 3_{10}-helical peptide as indicated by DMSO titration experiments.

Chapter 3: Section A: Consequences of "Disallowed" Conformations on Constrained β-Turn Peptides

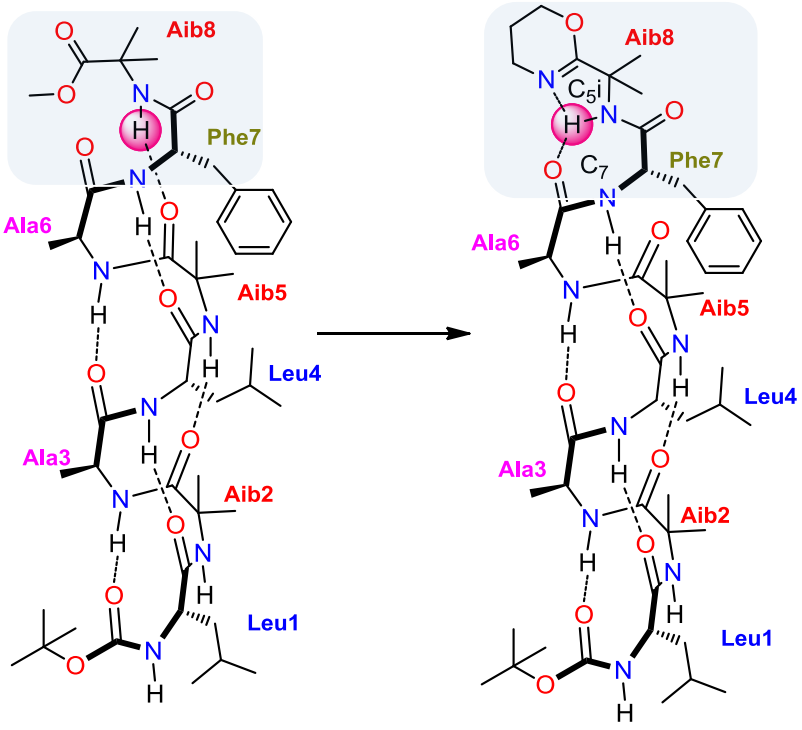
Here we are describe the consequence of disallowed conformations the on a C-terminus of a type-II β-turn. We choose stereochemically constrained Type-II β-turn Pro-Aib dipeptide analogue which is the ideal model to mirror the structural effects of introducing the A→I modification at the C-terminus. The imidate containing peptidomimetic crystallised in dichloromethane and hexane mixture. Analysis of crystal structure revealed that Aib NH is involved in 3-centered H-bonding interactions with the N of oxazine and N of proline. This constrains Aib in a conformation that is natively disallowed to it. The (ϕ, ψ) angles of Aib residue fall in the (180,0) region which is strictly disallowed for natural peptides due to steric clashes involving the back bone amide N-H. More importantly there are two C^{β}•••O interactions which are accomidated in the crystal structures. Both oxygen's were place in staggered orientation of the Pro oxygen (O_{Pro}) between the two β-CH_3 groups of Aib, which is again strictly disallowed in natural peptides due to strong C^{β}•••O_{i-1} hard sphere clashes. However no vdW space violations are observed between these atoms.



Chapter 3: Section B: Conformational Effects of "Disallowed" Aib on a 3_{10}-Helical peptide

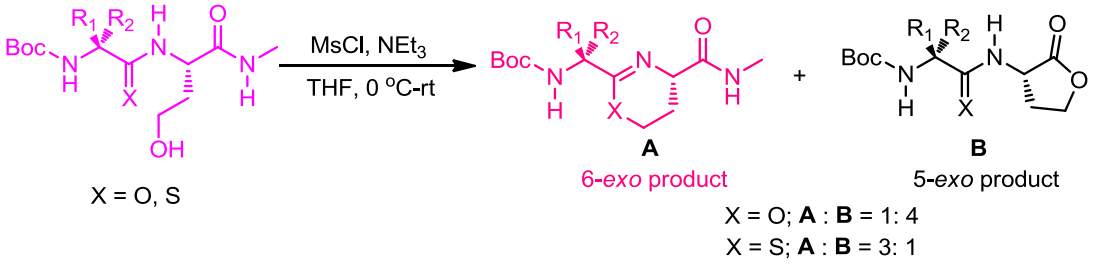
In order to investigate the origins and consequences of "disallowed" conformations on a folded helical peptide body, the conformationally stable peptide sequence Boc-Leu1-Aib2-Ala3-Leu4-Aib5-Ala6-Phe7-Aib8-OMe (**3_{10}-helix-OMe**) was chosen which is known to adopt 3_{10}-helix in crystal structure. Analyses of the ROESY spectra, DMSO

titration experiments, and CD spectra of **3_{10}-helix-OMe** and its **Oxa** analogue reveal that their solution conformations are identical to those of the crystal structure of **3_{10}-helix-OMe**. Six sequential i+3→i intramolecular backbone H-bonds stabilize the 3_{10}-helical peptide fold in both peptides in solvents of varying polarity. The N-terminal and central segments of the helical molecules are quite structurally rigid and are not deformed. The presence of the disallowed Aib*8 residue in **Oxa** analogue has a clear conformational effect mainly on the residue Phe7. It looks like the Phe7 amide H is involved in shielding, the Aib*8 amide H through a bifurcated hydrogen bonding interactions with the nitrogen of oxazine and carbonyl oxygen of Ala6 residue. Maximum structural distortion effect on the registers closest to the putative imidate bond. Our results show that "disallowed folds need not denature order in the peptide fold".

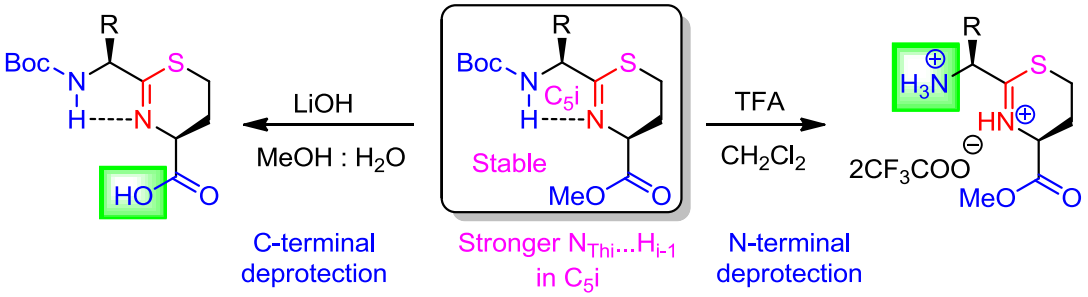


Chapter 4: Synthetic Methods for Introducing the A → I Modification anywhere along the Peptide Chain

Here we describe the incorporation of imidate isostere in the middle of any peptide sequence.

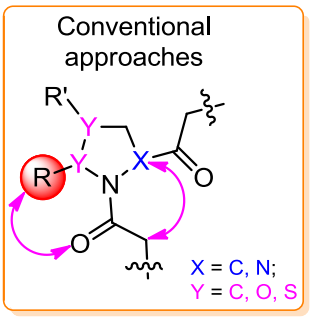


In **Oxa** selectivity is towards 5-*exo*-cyclo-*O*-alkylation in 1 : 4. In **Thi** it is towards 6-*exo*-cyclo-*S*-alkylation in 3 : 1 ratio. This is because of better nucleophile of sulphur (S). We saw that **Thi** is stable to peptide coupling, N- and C- terminus protection, deprotection conditions and can be easily incorporated in middle of peptide.



Chapter 5: Section A: *Cis-trans* Isomerism in the X-Pro Peptide Bond

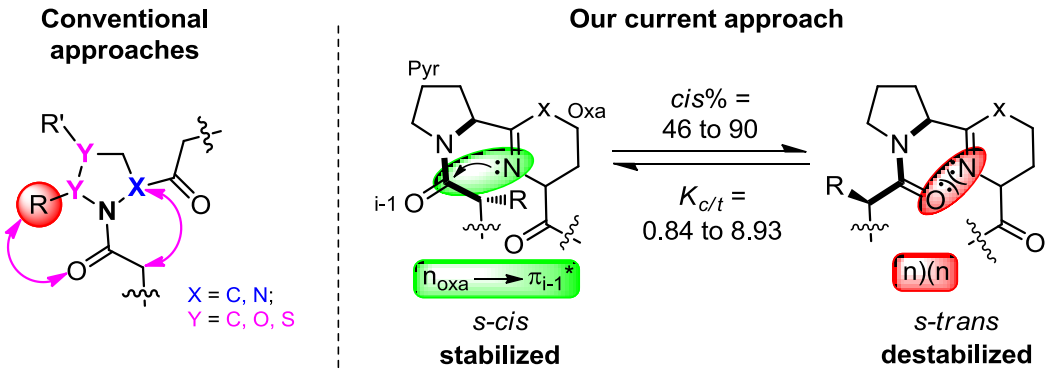
In tertiary amides like X-Pro peptides having high propensity to access *cis* conformations due to similar environment in both *cis* and *trans* around the C^{α} of X. X-Pro peptide bonds, constrained in *s-cis* conformations are prevalently found in the turn regions of peptides with the residue 'X' in the i+1th position and Pro at the i+2nd position of the *β*-turn. These types of turns are termed as the type VI *β*-turns. For better understanding of the molecular recognition at specific *cis* X-Pro peptide bonds in biological events, the structure and dynamics of various constrained *cis* X-Pro peptide bond analogues with varying steric and electronic perturbations have been studied. Many models have been developed for stabilizing *cis* conformer by perturbation of molecular recognition surface of proline by employing steric and electronic interaction. In biological functions proline molecular recognition surface and *cis* X-Pro peptide bond more important. There is need of novel method for stabilizing X-Pro peptide bond in *cis* conformer without modifying the pyrolidine ring in proline.



Chapter 5: Section B: Biasing the *cis/trans* Equilibrium in X-Pro Peptides using Reverse $n_i \rightarrow \pi_{i-1}^*$ Interactions

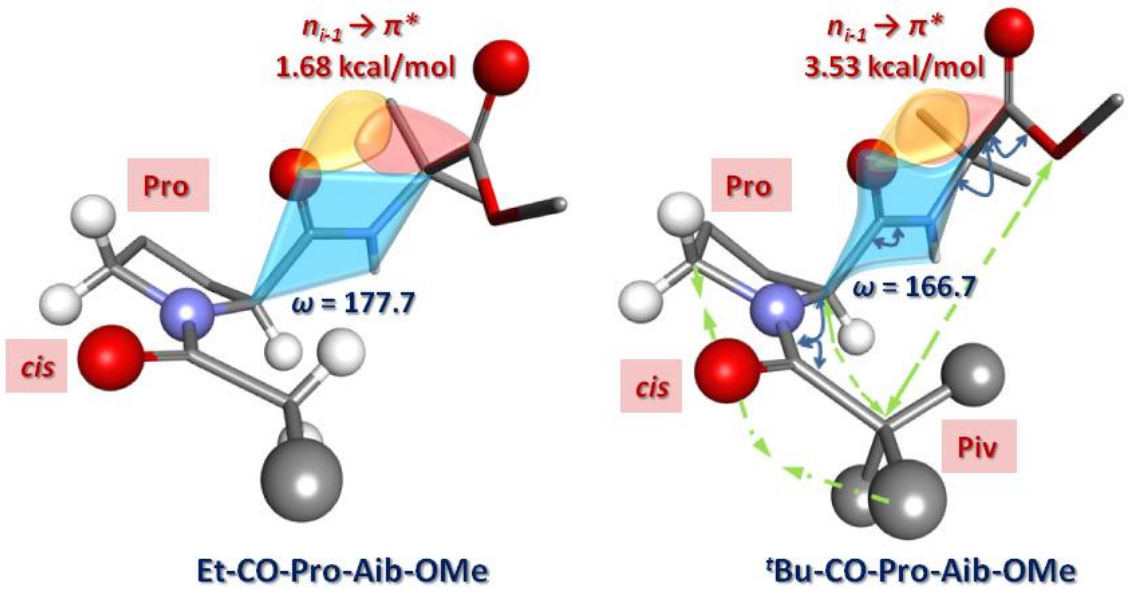
Here we present our findings that peptidomimetics containing the 5,6-dihydro-4*H*-1,3-oxazine (Oxa) and 5,6-dihydro-4*H*-1,3-thiazine (Thi) functional groups at the C-terminus of Pro selectively and remotely stabilize the *s-cis* rotamers of the preceding pyrrolyl

(Xaa-Pro) 3° amide bonds, while conserving these recognition elements. The *cis/trans* equilibrium of Xaa-Pro peptide bonds is shifted significantly in favor of the satirically disfavored *cis* isomers in these peptidomimetics (upto ~90%). We also provide evidence for the influence of an unusual $n \rightarrow \pi_{i-1}^*$ interaction in the *cis*, and the *n*)(*n* repulsion in the *trans*, conformers of these molecules to be at the origin of such *cis* stabilization.



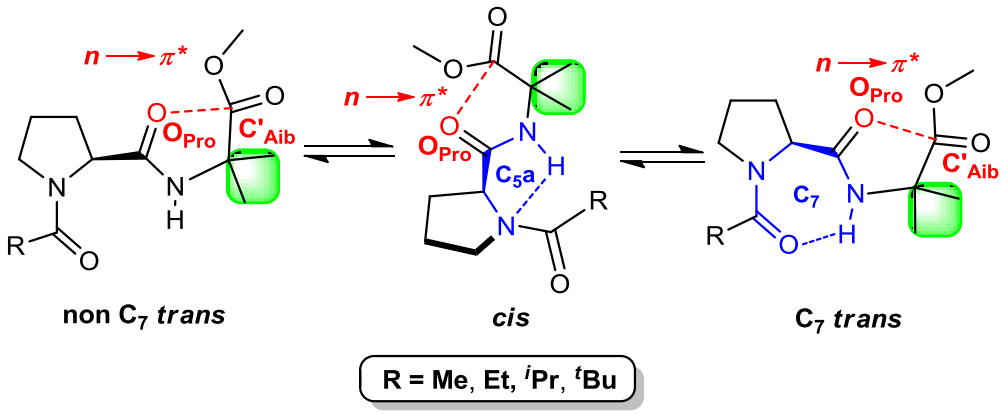
Chapter 6: Steric Interactions in the *cis* Piv-Pro Peptide Bond

The inaccessibility of *cis* Piv-Pro rotamer in any peptide is believed to be because the steric clashes between substituents on C^{α}_{X} and C^{α}_{Pro} are unavoidable in this conformer. Here we access the *cis* Piv-Pro conformer in crystal structure of Piv-Pro-Aib-OMe and show that it is sufficiently flexible to undergo bond distortions and avoid all steric clashes between substituents on C^{α}_{Piv} and C^{α}_{Pro}. It is however the unavoidable distortions in the dihedral angle of the Pro-Aib peptide bonds that destabilize the *cis* Piv-Pro conformer. The *cis* Piv-Pro conformer is indeed accessible, if such distortions are accommodated in the peptide.



Chapter 7: Steric and Electronic Interactions in the *cis* Isomer of Piv-Pro Peptide Bond in Solution

We have studied the electronic and steric interactions and the conformational equilibrium in two sets of homologous peptides, X-Pro-Aib-OMe (which contain Aib) and X-Pro-NH-Me, where X is acetyl, propionyl, isobutyryl and pivaloyl, in solvents of varying polarities consisting of carbontetrachloride, chloroform or dimethylsulfoxide, by means of their ^{1}H and ^{13}C-NMR, and FT-IR spectra. Formation of $n \rightarrow \pi^*$ interactions between the carbonyls that flank the Aib residue, influences the alleviation of steric interactions that are believed to preclude access to the *cis* conformer of the Piv-Pro peptide bond.

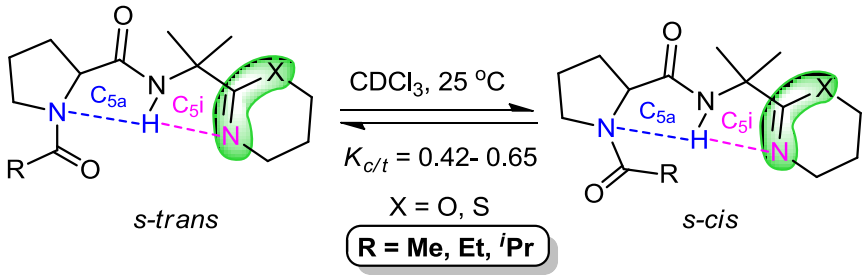


The *cis* Piv-Pro conformer is observable in the Aib containing peptides, at ambient conditions by FT-IR and at temperatures as low as 273 K by NMR. We estimate that the steric interactions contribute < 0.5 kcal/mol to the conformational free energy of X-Pro peptide bond isomerism, irrespective of the steric bulk on the acyl (X) group. The relative strengths of intramolecular hydrogen bonding interactions involving the X-Pro peptide motif in different conformers of these peptides influence their relative conformational stabilities.

Chapter 8: Remote Effect of Oxa and Thi Functional Groups on *cis-trans* Isomerism at X-Pro Peptide Bonds

The C_5a interaction at Pro residue occurs in the transition states for the intramolecular acid catalysis of *cis* → *trans* isomerization in peptidyl prolyl isomerases (PPIs) and enables the decrease in transition energy barrier for the isomerization process. We show that the N_{Pro}….H_{Aib} interactions in C_5a structures can be remotely effected in order to control in equilibrium constant values of the *cis/trans* isomerism ($K_{c/t}$) in X-Pro-

Aib-Oxa and Thi containing peptides. By this method we observed improvement in $K_{c/t}$ values from 0.18 in esters to 0.56 in Thi and 0.66 in Oxa containing peptides.



Analyses of the ROESY spectra, DMSO titration experiments, variable temperature experiments and FT-IR spectra of **R-CO-Pro-Aib-Oxa** (R = Me, Et, ^{i}Pr) and its **Thi** analogues reveals that both interactions (C_5a and C_5i) are persistent in *cis* and *trans* conformers of this peptidomimetics.